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10/731,379	12/09/2003	Daniel Zamanillo Castanedo	P03,0588	4441

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SCHIFF HARDIN, LLP  
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EXAMINER

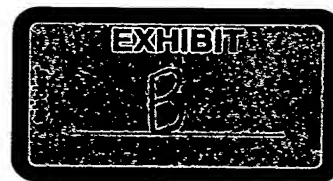
HIRIYANNA, KELAGINAMANE T

ART UNIT PAPER NUMBER

1633

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Please find below and/or attached an Office communication concerning this application or proceeding.



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**Office Action Summary**

Application No.

10/731,379

Applicant(s)

CASTANEDO ET AL.

Examiner

Kelaginamane T. Hirianna

Art Unit

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-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

- 1) ☒ Responsive to communication(s) filed on 09 December 2003.
- 2a) ☐ This action is **FINAL**. 2b) ☒ This action is non-final.
- 3) ☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

- 4) ☒ Claim(s) 1-29 is/are pending in the application.
- 4a) Of the above claim(s) \_\_\_\_\_ is/are withdrawn from consideration.
- 5) ☐ Claim(s) \_\_\_\_\_ is/are allowed.
- 6) ☒ Claim(s) 1-29 is/are rejected.
- 7) ☐ Claim(s) \_\_\_\_\_ is/are objected to.
- 8) ☐ Claim(s) \_\_\_\_\_ are subject to restriction and/or election requirement.

**Application Papers**

- 9) ☐ The specification is objected to by the Examiner.
- 10) ☐ The drawing(s) filed on \_\_\_\_\_ is/are: a) ☐ accepted or b) ☐ objected to by the Examiner.  
Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).  
Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).
- 11) ☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

- 12) ☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).
- a) ☐ All b) ☒ Some \* c) ☐ None of:
1. ☒ Certified copies of the priority documents have been received.
  2. ☐ Certified copies of the priority documents have been received in Application No. \_\_\_\_\_.
  3. ☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

\* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

- 1) ☒ Notice of References Cited (PTO-892)
- 2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
- 3) ☒ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08)  
Paper No(s)/Mail Date 01/21/2005.

- 4) ☐ Interview Summary (PTO-413)  
Paper No(s)/Mail Date. \_\_\_\_\_
- 5) ☐ Notice of Informal Patent Application (PTO-152)
- 6) ☐ Other: \_\_\_\_\_

## DETAILED ACTION

Claims 1-29 are pending and are presently under examination.

### *Specification*

Priority date for this invention, applied under 35 USC §119 (a-d) for the Foreign application SPAIN 200202815 filed on 12/09/2002.

### *Claim Rejections - 35 USC § 101*

35 U.S.C. 101 reads as follows:

Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 1-8, 14-20, 22-29 are rejected under 35 U.S.C. 101 because the claimed invention is not supported by either a specific or substantial asserted utility or a well established utility.

Claim 1-8, 14-20, 22-29 are also rejected under 35 U.S.C. 112, first paragraph. Specifically, since the claimed invention is not supported by either a specific or substantial asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention. According to the Revised Utility Examination Guidelines, see the Federal Register, Vol.66, No. 4, pp.1092-1099 (January 5, 2001), also available as repeated below from <http://www.uspto.gov/web/menu/utility.pdf>, the following definition of credible, specific and substantial utility.

"Credible Utility" - Where an applicant has specifically asserted that an invention has a particular utility, that assertion cannot simply be dismissed by Office personnel as being "wrong". Rather, Office personnel must determine if the assertion of utility is credible (i.e., whether the assertion of utility is believable to a person of ordinary skill in the art based on the totality of evidence and reasoning provided). An assertion is

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credible unless (A) the logic underlying the assertion is seriously flawed, or (B) the facts upon which the assertion is based is inconsistent with the logic underlying the assertion. Credibility as used in this context refers to the reliability of the statement based on the logic and facts that are offered by the applicant to support the assertion of utility. A credible utility is assessed from the standpoint of whether a person of ordinary skill in the art would accept that the recited or disclosed invention is currently available for such use. For example, no perpetual motion machines would be considered to be currently available. However, nucleic acids could be used as probes, chromosome markers, or forensic or diagnostic markers. Therefore, the credibility of such an assertion would not be questioned, although such a use might fail the specific and substantial tests (see below).

"Specific Utility"- A utility that is specific to the subject matter claimed. This contrasts with a general utility that would be applicable to the broad class of the invention. For example, a claim to a polynucleotide whose use is disclosed simply as a "gene probe" or "chromosome marker" would not be considered to be specific in the absence of a disclosure of a specific DNA target. Similarly, a general statement of diagnostic utility, such as diagnosing an unspecified disease, would ordinarily be insufficient absent a disclosure of what condition can be diagnosed.

"Substantial utility" - a utility that defines a "real world" use. Utilities that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use are not substantial utilities. For example, both a therapeutic method of treating a known or newly discovered disease and an assay method for identifying compounds that themselves have a "substantial utility" define a "real world" context of use. An assay that measures the presence of a material which has a stated correlation to a predisposition to the onset of a particular disease condition would also define a "real world" context of use in identifying potential candidates for preventive measures or further monitoring. On the other hand, the following are examples of situations that require or constitute carrying out further research to identify or reasonably confirm a "real world" context of use and, therefore, do not define "substantial utilities":

A. Basic research such as studying the properties of the claimed product itself or the mechanisms in which the material is involved.

B. A method of treating an unspecified disease or condition. (Note, this is in contrast to the general rule that treatments of specific diseases or conditions meet the criteria of 35 U.S.C. 101.)

C. A Method of assaying for or identifying a material that itself has no "specific and/or substantial utility".

D. A method of making a material that itself has no specific, substantial, and credible utility.

E. A claim to an intermediate product for use in making a final product that has no specific, substantial, and credible utility.

Note that "throw away" utilities do not meet the tests for a specific or substantial utility. For example, using transgenic mice as snake food is a utility that is neither specific (all mice could function as snake food) nor substantial (using a mouse costing tens of thousands of dollars to produce as snake food is not a "real world" context of use). Similarly, use of any protein as an animal food supplement or a shampoo ingredient are "throw away" utilities that would not pass muster as specific or substantial utilities under 35 U.S.C. ' 101. This analysis should, of course, be tempered by consideration of the context and nature of the invention. For example, if a transgenic mouse was generated with the specific provision of an enhanced nutrient profile, and disclosed for use as an animal food, then the test for ' specific and substantial asserted utility would be considered to be met.

"Well established utility" - a specific, substantial, and credible utility which is well known, immediately apparent, or implied by the specification's disclosure of the properties of a material, alone or taken with the knowledge of one skilled in the art. "Well established utility" does not encompass any "throw away" utility that one can dream up for an invention or a nonspecific utility that would apply to virtually every member of a general class of materials, such as proteins or DNA. If this is the case, any product or apparatus, including perpetual motion machines, would have a "well established utility" as landfill, an amusement device, a toy, or a paperweight', any

carbon containing molecule would have a "well established utility" as a fuel since it can be burned', any protein would have well established utility as a protein supplement for animal food. This is not the intention of the statute.

See also the MPEP § 2107 - 2107.02, and Brenner, Comr. Pts. V. Manson, 148 USPQ 689 (US SupCt 1966).

Instant claims are directed to a transgenic mouse and a method of making said mouse, and methods of using said mouse comprising a disruption in an endogenous Sigma 1 receptor gene, wherein the disruption is homozygous wherein the phenotype of mutant mouse was indistinguishable from wild type mouse with exception of a marginal insensitivity to (+)SKF-10047-induced hyperactivity assay.

The specification of the instant application discloses mice which have a homozygous disruption in endogenous the Sigma 1 receptor gene and absence of a overt phenotypic difference in said mutant mouse relative to wild type controls except for a marginal insensitivity to (+)SKF-10047-induced activity assay. Phenotype does not correlate well with any of the art-anticipated role of Sigma receptor proteins. The art teaches that the members of Sigma receptor family are associated with various physiological and pathological conditions including CNS pathologies such as schizophrenia, amnesia and learning and memory deficits as well as other types of psychiatric disorders, (Maurice et al., 1997, Prog. Neuro Psychopharmacol and Biol. Psychiat. 21:601-402, see abstract; Prasad et al., 1998, J. Neurochem. 70:443-451, see abstract). The specification fails to provide any nexus between the asserted phenotype and any disease or conditions that would enable one skilled in the art to use the invention as claimed.

Thus at the time of filing of the instant application, the artisan would not have found claimed utilities evident because the art is devoid of any teachings for the role of specific function for the sigma receptor I gene other than the ones anticipated as above for the members of sigma receptor family. Since the mice disrupted Sigma I receptor homozygous mice are not specific to any one disease or condition, the artisan, at the time of filing, would not know how to use the mice or any data resulting from using the mice for substantial and specific purpose.

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As set forth in the utility guidelines above, a general statement of therapeutic utility for an unspecified disease is non-specific, renders the purported utility of the claimed mice to be non-specific. The usefulness of the mutant mice, as models for disease, is not clear absent the assessment that they reflect a particular disease state. This leaves the skilled artisan to speculate the uses of the mice, cells, and methods, as claimed. Under the utility guidelines set forth above, requirement for further research or experimentation renders the claimed invention as lacking in a specific or substantial utility.

Utilities that require or constitute carrying out further research to identify or reasonably confirm a "real-world" context of use are not considered substantial utilities. The evidence of record has not provided any other utilities for the transgenic mice encompassed by the claims that are substantial and specific. Since the mice have no determined specific function, the relation to any disease or condition is unknown, and further, because the phenotypes in the disrupted Sigma receptor I-homozygous mice are not specific to any one disease or condition, the artisan, at the time of filing, would not know how to use the mice or any data resulting from using the mice. To make such a determination, the skilled artisan would need to further research the mice, to determine if functions associated with Sigma receptor I are present in the mice, and then identify disease or conditions associated with the disclosed phenotypes. None of the asserted utilities of the transgenic mice comprising Sigma receptor I gene disruption, cells and tissues derived from the same, and methods of producing the same, appear specific and substantial, because they do not correlate to the art anticipated role/s of Sigma receptor I gene.

### ***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

"The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make

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and use the same, and shall set forth the best mode contemplated by the inventor of carrying out his invention."

Claim 1-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claims contain subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention

Instant claims are directed to a transgenic non-human mammal with a homozygous or heterozygous mutation comprising gene-disruptions in a gene belonging to the genus of endogenous Sigma receptors and to a cell or tissue obtained from the same and cultured in vitro, to a method or process of making a transgenic non-human mutant mammal comprising a disruption of an endogenous Sigma I receptor gene, to a targeting construct for disrupting the gene belonging to sigma receptor 1 & genes gene, to a method of using said mutant mouse for evaluating potentially useful compounds for treating or preventing a CNS disorder, stress conditions, to a method determining the effect of a compound on said non human-mammal or a cell derived from said mammal,

The scope of the claims encompasses any and/or all non-human mutant mammals (mouse, cat, whales, ... etc) that are deficient in any and/or all endogenous Sigma receptors (e.g. Sigma receptor 1-3 etc), any vector that could be used for homologous recombination for making said nonhuman-mutant mammals, any cell derived from said mammals. By the breadth of the generic claim for "an endogenous sigma receptor gene" the applicants attempt to encompass genes including coding sequences, regulatory sequences, introns etc of all the members of Sigma receptor family and all possible ways of obtaining a mutant non-human mammals with said mutations comprising genes disruptions.

At the best specification describes a single example of a mouse with mouse Sigma I gene disruption, a single method generating said mutant mouse, a single vector construct for generating said mouse (Example 1-2). The specification further limits to a conventional gene-targeted endogenous Sigma I receptor gene generated by a



conventional method of gene targeting and using a conventional gene targeting vector construct with an art established gene Sigma I receptor gene sequences (Example 1-2 and figures). The specification further describes Sigma I gene and protein expression in various organs of a heterozygous and homozygous mutant mice has been described (Example 2 and figures), binding assays and a behavioral evaluation is carried out. The only mentioned phenotypic difference between wild type mouse and the homozygous mutant mouse was with reference to a difference in hyperactivity activity assay in response to SKF-10047 (data is not shown in the specification).

Besides mouse the specification does not describe any other mutant non-human mammals that are disrupted in any sigma receptor genes, does not describe any targeting vectors other than a single one for a mouse sigma receptor gene, does not describe any other method of making a Sigma receptor mutant animal other than a art established conventional method of gene targeting, does not describe culturing any mutant cells or their useful properties, does not describe any of the broadly claimed methods of utilizing the mutant for evaluation of any useful compounds for preventing or treating any disorders of CNS or memory alteration or stress conditions or producing analgesia or neuro-protection, does not describe any method of utilizing Sigma receptor-2 other sigma receptor gene mutant non-human mammals for validating or developing any drugs for diagnosis or treatment of cancer or for preventing, reducing or alleviating any side effects due administration of any neuroleptic agents, does not sufficiently describe the methods of determining the effect of any compounds on broad claims to mutant non-human mammals or the cells derived from the same. Specification does not describe any Sigma receptor gene mutant non-human mammal possessing any significant phenotype that would enable the use of any said mutant non-human mammal to serve as a model for any art established human/animal diseases or human/animal conditions.

Since the disclosure fails to describe the common attributes or characteristics that identify representative number of members of the said genus non-human mammals or their significant phenotypes or the methods of making and using the same for the broad claims of utility as described above one skilled in the art would reasonably

conclude that the disclosure fails to provide a representative number of species to describe the genus. The claimed invention as a whole thus not adequately described in the specification and which is not conventional in the art as of applicants' effective filing date. Claiming all divergent species that achieve a result as contemplated by the application without defining the means and/or uses will do so not in compliance with the written description requirement. Rather, it is an attempt to preempt the future before it has arrived. "The written description requirement has several policy objectives. The essential goal of the description of the invention requirement is to clearly convey the information that an applicant has invented the subject matter which is claimed." In re Barker, 559 F.2d 588, 592 n.4, 194 USPQ 470, 473 n.4 (CCPA 1977). Another objective is to put the public in possession of what the applicant claims as the invention. See Regents of the University of California v. Eli Lilly, 119 F.3d 1559, 1566, 43USPQ2d 1398, 1404 (Fed. Cir. 1997), cert. denied, 523 U.S. 1089 (1998)."

To satisfy the written description requirement, a patent specification must describe the claimed invention in sufficient detail such that the Artisan can reasonably conclude that the inventor(s) had possession of the claimed invention. Such possession may be demonstrated by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and/or formulae that fully set forth the claimed invention. Possession may be shown by an actual reduction to practice, showing that the invention as claimed is "ready for patenting", or by describing distinguishing identifying characteristics sufficient to show that applicant was in possession of the claimed invention (January 5, 2001 Fed.Reg., Vo.66, No. 4, pp. 1099-11).

Claims 1-29 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a mouse a mouse with a Sigma I gene disruption carried out using a conventional method of gene targeting with a conventional gene targeting construct vector construct for mouse Sigma I receptor gene, does not provide enabled description of targeting of any other sigma receptor genes and in any other mammals besides mouse or their use. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

Since the specification fails to disclose any other mutant mammals with any sigma receptor genes, or their phenotypes or uses. The applicant's disclosure does not

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enable one skilled in the art to practice the invention as claimed without further undue amount of experimentation, which requires the construction and characterization of any and all mutant mammals with a disruption in any and all sigma receptors. At issue, under the enablement requirement of 35 U.S.C.112, first paragraph is whether, given the Wands-factors, the experimentation was undue or unreasonable under the circumstances. "Experimentation must not require ingenuity beyond that to be expected of one of ordinary skill in the art." See *Fields v. Conover*, 443 F.2d 1386, 170 USPQ 276 (CCPA 1970).

Claims 1-8 and 14-29 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

There are many factors to be considered when determining whether there is sufficient evidence to support a determination that a disclosure does not satisfy the enablement requirement and whether any necessary experimentation is "undue." These factors include, but are not limited to: (1) The breadth of the claims; (2) The nature of the invention; (3) The state of the prior art; (4) The level of one of ordinary skill; (5) The level of predictability in the art; (6) The amount of direction provided by the inventor; (7) The existence of working examples; and (8) The quantity of experimentation needed to make or use the invention based on the content of the disclosure. In *re Wands*, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988). All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below as to show that one of ordinary skill in the art has to go through "undue experimentation" in order to practice the invention.

***Nature of the invention:*** The invention relates to Sigma receptor mutant non-human mammals, method of generating said mutant non-human mammals and methods of utilizing said mutant non-human mammals and/or the cells derived from the same for evaluating compounds useful for treatment of CNS disorders and other conditions described above.

***Breadth of the claims And Guidance of the Specification*** and The scope and breadth of the instant claims, read in the light of instant specification and the state of the art at the time of filing encompass methods of making any non-human mutant mammal comprising a disruption in any and /or all Sigma receptor genes and utilizing said non-human mutant mammals with said mutation in any and/or all genes belonging to genus Sigma receptors for evaluating any and/or all compounds for their potential in preventing or treating any and/or all disorders of the CNS or memory alterations or stress conditions or drug addictions in any subject and further encompasses the use of said mutant non-human mammal in validating and developing drugs for diagnosis or treatment of any cancer or any degenerative processes in any subject and still further encompasses methods for preventing or reducing or alleviating any side effects associated the administration of any and/or all agents that are neuroleptic and methods for conducting any and/or all further biological research on sigma receptors.

With respect to instant claims the specification provides by means of specific examples guidance and/or evidences regarding a single example of a mouse with Sigma I gene disruption. specification further limits to a conventional gene-targeted endogenous Sigma I receptor gene generated by a conventional method of gene targeting and using a conventional gene targeting vector construct. The only mentioned phenotypic difference between wild type mouse and the homozygous mutant mouse was with reference to a difference in hyperactivity activity assay in response to SKF-10047 (data is not shown in the specification).

Specification does not enable any mutant non-human mammals that are disrupted in any sigma receptor genes, does not enable the Sigma 1 receptor targeted mouse as it lacks a substantial use, does not enable any sigma receptor targeted mutant cells as they lack a substantial use, does not enable any of the broadly claimed methods of utilizing the mutant for evaluation of any useful compounds for preventing or treating any disorders of CNS or memory alteration or stress conditions or producing analgesia or neuro-protection, does not describe any method of utilizing Sigma receptor-2 other sigma receptor gene mutant non-human mammals for validating or developing any drugs for diagnosis or treatment of cancer or for preventing, reducing or

alleviating any side effects due administration of any neuroleptic agents, does not provide sufficient enablement for the methods of determining the effect of any compounds on broad claims to mutant non-human mammals or the cells derived from the same. Specification does not describe any Sigma receptor gene mutant non-human mammal possessing any significant phenotype that would enable to serve as a model for any art established human/animal diseases or human/animal conditions.

The specification thus fails to provide an enabling disclosure for the full scope and breadth of the invention as claimed. In the absence of adequate description of the enabled invention commensurate with the breadth and scope of the claim one of ordinary skill in the art would conclude that the claimed invention is unpredictable and would require an undue amount of experimentation to practice the full scope of the same. Applicants' attention is drawn to *In re Shokal*, 242 F.2d 771, 113 USPQ 283 (CCPA 1957). The test is whether the species completed by applicants prior to the reference date or the date of the activity provided an adequate basis for inferring that the invention has generic applicability.

***The level of one of ordinary skill in the Art at the Time of Invention:*** The level of one of ordinary skill in the art at the time of filing of the instant application is high requiring an advanced degree or training in the relevant field. The status of the art at the time of filing was such that said skilled in the art would not have been able to make or use the invention for its fully claimed scope without undue experimentation.

***State of the Art, the Predictability of the Art:*** At about the effective filing date of the present application art does not provide enablement for making a gene disrupted gene knockout mutant non-human mammal other than in mouse species. Gene targeting method as described for a mouse Sigma I receptor gene as described in the instant invention is only enabled for mouse. Art is still unpredictable with regard to achieving a desired phenotype even in a mouse that is of substantial use or utility for example modeling human/non-human animal diseases or conditions. Holschneider et al. *Int J. Devl. Neuroscience* 18:615-618, 2001, state that knocking out or insertion of a single gene may result in no phenotypic change. This may be the case, in particular, if there exist gene redundancy mechanisms whose presence may prevent abnormal

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phenotypes from becoming expressed. Conversely, single genes are often essential in a number of different behaviors and physiologic processes. Silence, ablation of an individual gene may prove so drastic as to be lethal, or so widespread as to create an amalgam of phenotypes whose interpretation becomes confounded by the interactions of the various new physiologic changes or behaviors." (See p. 615, col. 1-21. Holschneider et al., discuss various factors that contribute to the resulting phenotype of transgenic mice, including compensatory systems which may be activated to mask the resulting phenotype, these compensatory changes may be due to the differential expression of another gene, which may be regulated by the downstream product of, the ablated gene, as well as the variability in phenotypic characterization due to particular mouse strains (see p. 616, 1<sup>st</sup> column. Even the conventional targeting approach, as is in the instant application, has limitations including functional redundancy of closely related proteins, induction of compensatory processes and early embryonic lethality (Holschneider et al., Int. J. Devl. Neuroscience 18:615-618, 2000; see p. 616, 1<sup>st</sup> col. 2<sup>nd</sup> ¶).

It is noted that specific claims are presented, which are directed to cells/tissues obtained from the transgenic mice (see claims 4 & 8). These claimed cells will not have the phenotypes claimed for the mice, and thus, it is unclear what or any phenotype these cells exhibit. Therefore, the contemplated uses of these cells, such as for screening any drugs/compounds is not enabling. The instant specification is not found to be enabling, because there is a lack of a significant phenotype in Sigma I receptor gene disrupted mouse of the instant invention that can be correlated with any known disease or disorders of humans or animals of economic interest.

There is no nexus between this phenotype and art contemplated functions of Sigma receptors such that one of skill in the art could make and use these mice in any of the contemplated uses as claimed. The art regarding the function of the Sigma receptors teaches that. In the light of unpredictability and lack of guidance in the art it requires undue experimentation for one skilled in the art to know the correlation, if any, exists between the observed phenotype of a marginal decrease in hypermotility in response to prozocine.

Furthermore, there is unpredictability in the art with regard to the resulting less significant phenotypes because of the possibility that such variations are due to differences genetic backgrounds of mouse strains used in breeding to generate the homozygous gene disrupted mice as it is in the instant invention. It is well established in the art, the homozygous mice claimed will have some 129/Sv genes, regardless of the outward appearance of the mice, due to the 129-knockout construct. F2 mice homozygous for the disrupted Sigma I receptor gene have genotypes from two parents, 129/Sv and C57BL/6 or 129Sv and CD-1 due to recombination events during gametogenesis (Gerlai, G. et al. Trends in Neuroscience. 19: 177- 181 (1996), specifically page 178, lines 1-5). These mice are genotypically different from wild-type littermates, and thus wild-type littermates are not good controls for the null mice (Gerlai, page 178, col. 1, lines 6- 18). This effect causes linkage disequilibrium between the transgene and surrounding genes, producing a "hitchhiking donor gene confound" (Lariviere et al. J. Pharmacology Experiment. Therapeut. 297: 467-473 (2001), particularly, page 468, col. 1, 2<sup>nd</sup> ¶, lines 1-4). To overcome the "hitchhiking effect, two remedies are suggested: testing a large number of mice (Gerlai, page 178, col. 2, lines 1-5) and many backcrosses (Lariviere, page 468, col. 1, 2<sup>nd</sup> ¶, line 18-21). However, even with a large testing population and multiple backcrossings, some of the 129/Sv1 genome will remain. Thus, the behavioral and physiological effects observed in the presently claimed-Sigma receptor 1 null mice could be due to 129/Sv1 gene background (Gerlai, page 179, col.1, lines 9-14). There is no way to tell given the tests in the disclosure. Further there does exist genetic heterogeneity with regard to lipoprotein levels in inbred strains of mice (see Cole et al., Metabolism, 1990, 39:155-160, p.155, 1<sup>st</sup> ¶, abstract)). Given this unpredictability and in the absence of guidance in the specification one of skill in the art will find it unsubstantial and hence unable to practice the invention for any specific purpose.

These claims are not enabled because one of skill in the art would not be able to produce any non-human mutant mammals, as instantly claimed, exhibiting consistent phenotype with art contemplated function for Sigma receptors, and further, one would not be able to use these mutant non human mammals for a substantial use, since they

do not exhibit these phenotypes. One of skill in the art would not be able to rely upon the state of the art in order to produce Sigma 1 mutant non-human mammal other than in mice and would not be able produce a mice with a phenotype for a substantial use as claimed, because, for reasons stated above, because of the unpredictability in the resulting phenotype of any particular knockout mouse. Accordingly, in view of the lack of teachings or guidance provided by the specification with regard to an enabled use for a mouse comprising a disruption in an endogenous Sigma receptor gene, absent guidance provided by the specification to overcome the art-recognized unpredictability regarding gene disrupted mice and lack of technology to produce other gene disrupted non-human mammals, and for the specific reasons cited above, it would have required undue experimentation for one of skill in the art to make and use the claimed invention.

***Claim Rejections - 35 USC § 103***

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

(a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negated by the manner in which the invention was made.

Claims 9-21 and 29 are rejected under 35 U.S.C. 103(a) as being unpatentable over Capecchi (U.S. Pat. No. 5,464,764) in view of Seth et al., (2000, Biochemical and Biophysical Research communications 241: 535-540).

The claims are directed to a homologous recombination vector for a Sigma receptor gene with a positive and negative selection marker and a first and second homology region of said gene, a host cell transfected with said vector, a non human mutant mammal deficient (targeted) endogenous Sigma receptor gene, a process of making a non-human mutant animal.

Capecchi teaches a vector to be used to produce knockout mice. Particularly, that the vector has a first and second segments of homologous DNA sequence, and a positive selection marker between the two homologous sequences. See Figure 1.



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Furthermore, they teach various markers that can be used in these vectors (Table 1, col.7-8). They teach that these vectors can then be used to produce transgenic animals, wherein ES cells are the target cells (Col. 15, lines 59-67), wherein the vector can then be introduced into the ES cells by electroporation or microinjection. These transformed ES cells can then be combined with a blastocysts and then grown and contribute to the germ line of the resulting chimeric animal (Col. 16, lines 1-10). They teach that cell lines from the animals can then be used to characterize gene function, or be used in assays (Col. 12-13, bridging paragraph). Capecchi clearly show that these vectors and methods can be used to determine the biological function of any known gene of interest. Capecchi however, does not teach the sequence for Sigma receptor gene.

Seth teaches that cDNA sequence of Sigma I receptor (Abstract, p.536). Known sequence, which would fulfill the limitations of the claims, because this sequence would be considered homologous to at least a portion of the endogenous Sigma receptor gene (p.538 and Figure 2).

Thus, it would have been obvious to one of ordinary skill in the art at the time of the instant invention to modify the homologous sequences in targeting vectors as taught by Capecchi with segments of DNA sequence for Sigma I receptor as taught by Seth to make a targeting construct and use the construct to generate a gene disrupted mouse, and further breed them to generate a homozygous gene disrupted mouse where the genome of the mouse comprises a homozygous disruption of a Sigma receptor I gene. One would have been motivated use the method making a targeting vector and for producing mice having a homozygous disruption of sigma receptor gene as they may provide a disease model for investigating the art described diseases or conditions associated with sigma receptors malfunctions. One would have a reasonable expectation of success of making and using Sigma receptor gene disrupted mouse as prior art provides the requisite teaching, suggestion and motivation to make and use the claimed invention as taught by Capecchi and Seth as above.

Thus, the claimed invention was *prima facie* obvious.


**Conclusion:**

No claim allowed.

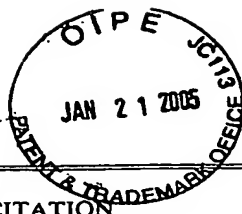
Any inquiry concerning this communication or earlier communications from the examiner should be directed to examiner *Kelaginamane Hiriyanne* whose telephone number is (571) 272-3307. The examiner can normally be reached Monday through Friday from 9 AM-5PM. Any inquiry concerning this communication or earlier communications regarding the formalities should be directed to Patent Analyst *William N. Phillips* whose telephone number is 571 272-0548. If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, *Dave Nguyen*, may be reached at (571) 272-0731. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300. Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see <http://pair-direct.uspto.gov>. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). When calling please have your application serial number or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. For all other customer support, please call the USPTO call center (UCC) at (800) 786-9199.

Kelaginamane T. Hiriyanne  
Patent Examiner

Art Unit 1633



SUMESH KAUSHAL, PH.D.  
PRIMARY EXAMINER



Sheet 1 of 1

Form PTO-1449

INFORMATION DISCLOSURE CITATION  
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10/731,379

Applicant

Daniel Zamanillo Castanedo, et al.

Filing Date

December 9, 2003

Group Art Unit

1632

## U.S. PATENT DOCUMENTS

Examiner's Initials	Document Number	Date	Name	Class	Subclass	Filing Date If appropriate
AA						
AB						

## FOREIGN PATENT DOCUMENTS

	Document Number	Date	Country	Class	Subclass	Translation	
						Yes	No
AC							
AD							

## OTHER PRIOR ART (Including Author, Title, Date, Pertinent Pages, Etc.)

KH	AE	Kaiser C., Pontecorvo M.J. & Mewshaw R.E. (1991) Sigma receptor ligands: function and activity. <i>Neurotransmissions</i> 7 (1): 1-5
KH	AF	Walker J.M., Bowen W.D., Walker F.O., Matsumoto R.R., De Costa B. & Rice K.C. (1990) Sigma receptors: biology and function. <i>Pharmacological Reviews</i> 42 (4): 355-402
KH	AG	Bowen W.D. (2000) Sigma receptors: recent advances and new clinical potentials. <i>Pharmaceutica Acta Helveticae</i> 74: 211-218
	AH	Hanner M., Moebius F.F., Flandorfer A., Knaus H.G., Striessing J., Kempner E. & Glossmann H. (1996) Purification, molecular cloning, and expression of the mammalian Sigma-i binding site. <i>Proceedings of the National Academy of Sciences USA</i> 93: 8072-8077
	AI	Kekuda R., Prasad P.D., Fei Y.-J., Leibach F.H. & Ganapathy V. (1996) Cloning and functional expression of the human type I Sigma receptor (hSigmaRI). <i>Biochemical and Biophysical Research Communications</i> 229: 553-558
	AJ	Seth P., Leibach F.H. & Ganapathy V. (1997) Cloning and structural analysis of the cDNA and the gene encoding the murine type I sigma receptor. <i>Biochemical and Biophysical Research Communications</i> 241: 535-540
	AK	Seth P., Fei Y.-J., Li H.-W., Huang W., Leibach F.-H. & Ganapathy V. (1998) Cloning and functional characterization of a receptor from rat brain. <i>Journal of Neurochemistry</i> 70: 922-931
	AL	Prasad P.D., Hui W.L., Fei Y.-J., Ganapathy M.E., Fujita T., Plumley L.H., Yang-Feng T.-L., Leibach F.-H. & Ganapathy V. (1998) Exon-intron structure, analysis of promoter region, and chromosomal localization of the human Type I receptor gene. <i>Journal of Neurochemistry</i> 70: 443-451
	AM	Crane MS (1999) Mutagenesis and cell transformation in cell culture. <i>Methods Cell Sci.</i> 21(4):245-253
	AN	Earnest D.J., Liang F.Q., DiGiorgio S., Gallagher M., Harvey B., Earnest B., Seigel G. (1999) Establishment and characterization of adenoviral E1A immortalized cell lines derived from the rat suprachiasmatic nucleus. <i>J. Neurobiol.</i> Apr; 39(1):1-13
	AO	Schwartz B., Vicart P., Delouis C., Paulin D. (1991) Mammalian cell lines can be established <i>in vitro</i> upon expression of the SV40 large T antigen driven by a promoter sequence derived from the human vimentin gene. <i>Biol. Cell.</i> 73(i):7-14
	AP	Frederiksen K., Jat P.S., Valtz N., Levy D., McKay R. (1988) Immortalization of precursor cells from the mammalian CNS. <i>Neuron.</i> Aug; 1(6):439-448
	AQ	Nagy A, Rossant J, Nagy R, Abramow-Newerly W, Roder JC (1993) Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. <i>Proc Natl Acad Sci U S A</i> 90: 8424-8
	AR	Kaestner KH, Montoliu L, Kern H, Thulke M & Schutz G (1994) "Universal $\beta$ -galactosidase cloning vectors for promoter analysis and gene targeting". <i>Gene</i> 148: 67-70
	AS	Kaestner KH, Hiemisch H, Schutz G. Targeted disruption of the gene encoding hepatocyte nuclear factor 3 gamma results in reduced transcription of hepatocyte-specific genes. <i>Mol Cell Biol.</i> 1998 Jul; 18(7):4245-51
	AT	Capecchi MR. The new mouse genetics: altering the genome by gene targeting. <i>Trends in Genetics</i> 1989 Mar; 5(3):70-6
	AU	Tybulewicz VL, Crawford CE, Jackson PK, Bronson RT, Mulligan RC. Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl proto-oncogene. <i>Cell.</i> 1991 Jun 28; 65(7): 1153-63
	AV	DeHaven-Hudkins D.L., Fleissner LC., Ford-Rice, F.Y. (1992) Characterization of the binding of [3H]-Pentazocine to sigma recognition sites in guinea pigs brain. <i>European Journal of Pharmacology</i> 227:371-378

Examiner /kelaginamane Hiriyanna/

Date Considered

04/20/2006

\*EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant.

**Notice of References Cited**

Application/Control No.

10/731,379

Applicant(s)/Patent Under  
Reexamination  
CASTANEDO ET AL.

Examiner

Kelaginamane T. Hiriyanne

Art Unit

1633

Page 1 of 1

**U.S. PATENT DOCUMENTS**

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
*	A	US-5,464,764	11-1995	Capecchi et al.	435/6
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

**FOREIGN PATENT DOCUMENTS**

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	N					
	O					
	P					
	Q					
	R					
	S					
	T					

**NON-PATENT DOCUMENTS**

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Seth et al., 2000, Biochemical and Biophysical Research communications 241: 535-540.
	V	
	W	
	X	

\*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)  
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# Cloning and Structural Analysis of the cDNA and the Gene Encoding the Murine Type 1 Sigma Receptor

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We have isolated a mouse cDNA which codes for a functional type 1 sigma receptor. The functional identity of the cDNA was established by expressing the cDNA in mammalian cells and measuring the cDNA-induced binding of haloperidol. Using this cDNA as the probe, we have isolated a murine genomic clone which contains the type 1 sigma receptor gene in its entirety. We have sequenced the gene completely, deduced the exon-intron organization and analyzed the promoter region sequence for transcription factor binding sites. The gene (~7 kbp) is TATA-less but contains CCAATC and GC boxes immediately upstream of the transcription start site. The gene consists of 4 exons and 3 introns. The 5'-flanking region contains putative binding sites for AP-1, AP-2, GATA-1 and steroid receptors. © 1997 Academic Press

Sigma receptors, which are expressed in various tissues of the nervous, endocrine, and immune systems, have been implicated in a number of physiological functions (1-4). Abnormalities in these receptors have been proposed to be the possible underlying causes of psychiatric disorders (1-4). Sigma receptors consist of several subtypes which are distinguishable by biochemical and pharmacological means (5). Among these subclasses, the type 1 receptor is the most well studied. It interacts with endogenous steroid hormones such as progesterone and testosterone (6-8) and its interaction with certain ligands is allosterically modulated by the antiepileptic drug phenytoin (9,10). Recent work on successful cloning of the type 1 sigma receptor from guinea pig liver (11) has advanced our understanding of the molecular nature of this particular subclass. Subsequently, we were successful in elucidating the primary structure of the human type 1 sigma receptor (12). In this paper,

we report on the cloning and structural analysis of the cDNA and the gene encoding the murine type 1 sigma receptor. The information on the structure, exon-intron organization and nature of the promoter region of the murine type 1 sigma receptor gene reported here is likely to be useful in the development of transgenic and knock-out animal models for understanding the pathophysiology of the receptor function.

## METHODS AND MATERIALS

**Isolation of the murine type 1 sigma receptor cDNA and its functional expression.** A cDNA library was constructed in pSPORT vector using poly(A)<sup>+</sup> RNA isolated from mouse kidney as described previously (13,14). The Superscript Plasmid System (Life Technologies, Inc.) was used for this purpose. A cDNA probe derived from the coding region of the rat type 1 sigma receptor cDNA (P. Seth *et al.*, submitted for publication) was used for screening the library. Sequencing of the cDNA insert of the positive clone was done by primer walking using an automated DNA sequencing system. The cDNA was functionally expressed in HeLa cells by the vaccinia virus expression technique (12). [<sup>3</sup>H]Haloperidol (sp. radioactivity, 15 Ci/mmol) was used as the ligand for the establishment of the function of the cloned cDNA. Equilibrium binding of the radioligand to the cell lysate was measured in 5 mM K<sub>2</sub>HPO<sub>4</sub>-KH<sub>2</sub>PO<sub>4</sub> buffer, pH 7.5 for 3 h in the absence or presence of unlabeled sigma receptor-specific ligands.

**Isolation and structural analysis of the murine type 1 sigma receptor genomic clone.** A mouse (129Sv strain) genomic DNA library in Lambda Dash II vector (Stratagene) was screened using the murine type 1 sigma receptor (mSigmaR1) cDNA as the probe. A positive phage colony was isolated as a single plaque after tertiary screening and the DNA insert in the clone was characterized by restriction mapping and Southern blot analysis. Digestion of the phage DNA from the positive clone with *Sac*I released the full-length DNA insert of ~50 kbp. Upon digestion with *Sac*I, several fragments were released, of which only a 7 kbp fragment hybridized to the full length mSigmaR1 cDNA. This insert was subcloned in pBluescript vector for sequence and structural analyses. Nucleotide sequence analysis was done using the GCG software from GenBank (15). The gene sequence was aligned with the sequence of the cloned mSigmaR1 cDNA to determine the splice junctions.

**5' Rapid amplification of cDNA ends (5' RACE).** To determine the transcription start site, we carried out 5' RACE using a commercially available kit (Life Technologies, Inc.) according to the manufacturer's protocol. Poly(A)<sup>+</sup> RNA isolated from mouse kidney was re-

<sup>1</sup> Corresponding author. Fax: (706) 721-6608.

GenBank accession numbers for the cDNA sequence and the gene sequence reported here are AF 030198 and AF 030199, respectively.

```

GGTGCCAGGCTGCCCCGCTAGAAATGCCGTGGGCCGCGGGACGGCGGTGGGCATGGATCACC
      M P W A A G R R W A W I T

CTGATTCTGACTATTATCGCAGTGTGATCCAGGCCGCTGGTTGTGGCTGGGCACTCAA
L I L T I I A V L I Q A A W L W L G T Q
      Exon1 ← ↓ → Exon2
AACTTCGCTTCTCTAGAGAAGAAATAGCGCAGCTTGCTCGACAGTATGCGGGGCTGGAC
N F V F S R E E I A Q L A R Q Y A G L D

CATGAGCTTGCCTTCTCTCGGCTGATCGTGGAGCTGCGGAGGCTGCACCCAGGCCACGTG
H E L A F S R L I V E L R R L H P G H V

CTGCCGGATGAGGAGCTGCAGTGGGTATTTGTGAACGCGGGCGGCTGGATGGGCGCCATG
L P D E E L Q W V F V N A G G W M G A M

TGTATTCTGCACGCCCTCGCTGTCTGAGTACGTGCTGCTCTTCGGCACCGCCCTGGGCTCC
C I L H A S L S E Y V L L F G T A L G S
      Exon2 ← ↓ → Exon3
CATGGCCATTTCGGGACGATACCTGGGCTGAGATTTCTGACACCATCATCTCTGGCACCTTC
H G H S G R Y W A E I S D T I I S G T F
      Exon3 ← ↓ → Exon4
CACCAATGGAAAGAGGGCACCACGAAAGTGAGGTCTTCTACCCAGGAGAGACAGTTGTA
H Q W K E G T T K S E V F Y P G E T V V

CACGGGCTTGGAGAAGCAACGGCTCTGGAGTGGGGACCAACACGTGGATGGTGGAGTAC
H G P G E A T A L E W G P N T W M V E Y

GGCCGGGTGTTATTCCGTCTACCCCTGTTCTTTGCACTAGCCGACACTTTCTTCAGCACC
G R G V I P S T L F F A L A D T F F S T

CAGGACTACCTCACACTCTTCTATACCCCTTCGGGCCTATGCCCGGGGCTCCGGCTTGAG
Q D Y L T L F Y T L R A Y A R G L R L E

CTTACCACCTACCTCTTTGGCCAAGACTCCTGACCAGCCAGGCCTGAAGGAAGATCTGTG
L T T Y L F G Q D S *

GATGGACAGGAGCGCGGCAGGACCACATCCATCTCTCTACTCGCTGGAACATCATATTTCAG
ACAGCAGCGTATACCATGCAGATATTAAGTACCTGCTGTATGAGGAAGGACATACATACT
TGTACATCCAGACACAGCTCTTGGGAAGCAAATGAGACACAGAGATGCTAAGACTTGTAT
GTACTGTAAGATCATGCACTCACATCCACCCAGAAAGGGTCCCTCTACATATACTCCAGGG
GGCCAGTCATGTTTGAACACACATCACAAGCTTGACTCACTGACTCAGGCTTTCCCTAGAG
CCTCCTGCTGGCATTCAGGGCTCTGGGATCAAGGGTTGAGGTAGATGTTACACACTACCT
CACTCTGATGCCTCATTCCTGTAGTAATCTCTGGTGAAGGGAAAGAGGAGCTGCCCTTCG
GAGGTCCCTTCACCTGCAGCTATGATGCCCTTCCCTTCATCCCTTTCCCTCACCATATGC
CTTATCTCCATTCCACTCCCTGCTATGCAATGCCCTTGGCTTGTCTCCCTGCCCTCC
CCCCCCCCGGCAACCAAGTTCAAGCTGGGAAAGCAGAGGCATGTAGAGGATGCTAAAGGGAA
GGGAGGTCCCTCACTACGGAGACCCTTGTGTGGGTTAGAAATGGGAAAGCTGGCTAGTGCC
CCAGGGCCAGTTCCCAAGGAGAGGACCTGGAGAAGTAAGCCGGGGTCCAAGGAGCCACCC
CATTCCAAAAGAAGCCTTTAGTGTGTGTGTGCACACCCTATCAGTTTCCAGACATATCTG
TTGCAATCTCTGTCTTCTGTCTCTCTCTGTTAATAAACAATATTAAACAAAAA
AAAAAA

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FIG. 1. Nucleotide sequence and predicted amino acid sequence of the mouse type 1 sigma receptor cDNA. The putative transmembrane domain is boxed and the polyadenylation signal is underlined. The positions of the splice junctions are indicated.

verse-transcribed with Superscript RNase H reverse transcriptase at 42°C using a primer corresponding to bases 429-446 of the cloned mSigmaR1 cDNA. Following this reaction, excess nucleotides and the primer were removed on a GlassMax spin column and the purified cDNA was homopolymeric-tailed with dCTP using terminal deoxynucleotidyl transferase. The resulting oligo(dC)-tailed cDNA was amplified by PCR using an abridged anchor primer provided in the 5'-RACE kit and a nested mSigmaR1-specific primer (bases 402-419). The PCR product was subjected to further amplification using a universal amplification primer, provided in the 5'-RACE kit and the same nested mSigmaR1-specific primer. The final PCR product was digested with *SaI* (a site present in the universal amplification primer used in the second round PCR) and *PstI* (a site present up-

stream of the position of the nested mSigmaR1-specific primer) and cloned in pSPORT vector for sequence analysis.

## RESULTS AND DISCUSSION

*Structural features of the mouse SigmaR1 cDNA.* The mouse SigmaR1 cDNA isolated from a mouse kidney cDNA library is 1,567 bp long (GenBank accession no. AF 030198) and contains a 672 bp long open reading frame (including the termination codon) (Fig. 1). This coding sequence is flanked by a 21 bp long 5'-untrans-

TABLE 1  
Functional Identity of the Cloned Mouse SigmaR1 cDNA

Inhibitor	pSPORT	pSPORT- mSigmaR1 cDNA	cDNA-specific
<sup>3</sup> H]-Haloperidol binding (pmol/10 <sup>6</sup> cells)			
None	1.40 ± 0.07 (100)	4.33 ± 0.02 (100)	2.93 ± 0.02 (100)
Haloperidol	0.31 ± 0.04 (22)	0.31 ± 0.01 (7)	0.01 ± 0.01 (1)
Clorgyline	0.22 ± 0.01 (15)	0.30 ± 0.02 (7)	0.08 ± 0.01 (3)
DTC	0.38 ± 0.03 (27)	0.96 ± 0.03 (22)	0.58 ± 0.03 (19)
(+)-3-PPP	0.52 ± 0.12 (37)	0.58 ± 0.01 (13)	0.06 ± 0.01 (2)

HeLa cells, transfected with either pSPORT or pSPORT-mSigmaR1 receptor cDNA, were lysed and the lysates were used for measurement of equilibrium binding (3 h incubation) of [<sup>3</sup>H]-haloperidol (5 nM). Concentration of inhibitors was 10 μM. Values in parentheses are percent of respective controls. DTC, 1,3-di-(2-tolyl)guanidine; (+)-3-PPP, (+)-3-(3-hydroxyphenyl)-N-(1-propyl)-piperidine.

lated region and a 874 bp long 3'-untranslated region. The cDNA contains a poly(A)<sup>+</sup> tail and the polyadenylation signal (AATAAA). The predicted protein consists of 223 amino acids with a single putative transmembrane domain, as indicated by hydropathy analysis of the protein sequence using the criteria of Eisenberg *et al.* (16). The amino acid sequence of the mouse type 1 sigma receptor is highly homologous to the sequences of the previously cloned guinea pig (87% identity; 91% similarity), human (90% identity; 93% similarity), and rat (92% identity; 96% similarity) type 1 sigma receptors. The amino acid sequence motif MPWAAAGR, a putative signal for retention in the endoplasmic reticulum (17), is present in the mouse type 1 sigma receptor as is the case with the human, rat, and guinea pig homologs.

*Functional identity of the mSigmaR1 cDNA.* To establish the functional identity of the cloned mouse

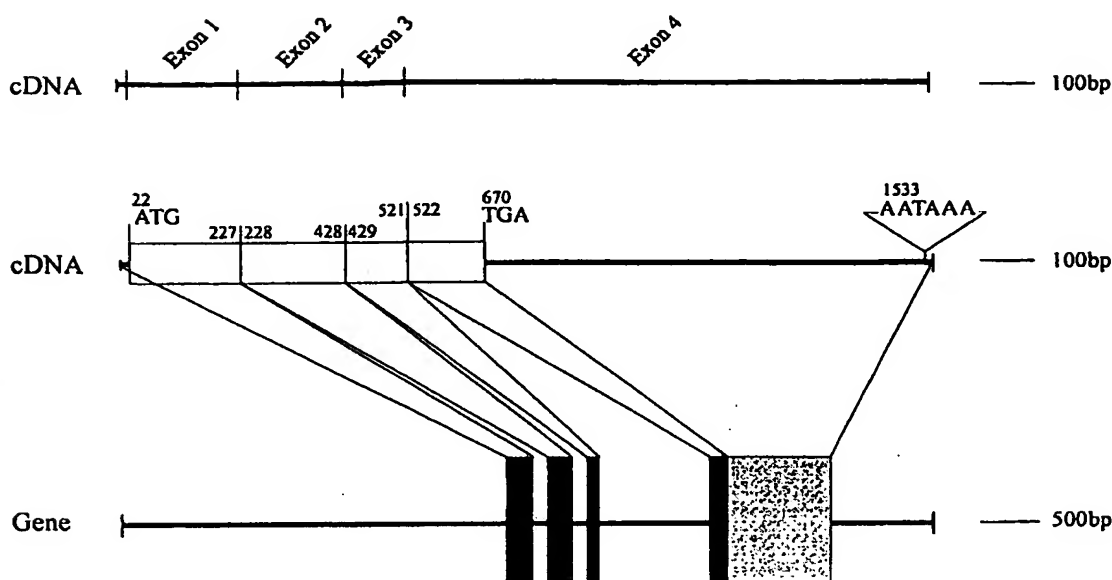
SigmaR1 cDNA, the cDNA was expressed in HeLa cells using the vaccinia virus expression technique (12). HeLa cells transfected with empty pSPORT vector served as controls. Sigma receptor function was monitored by measuring equilibrium binding of [<sup>3</sup>H]-haloperidol to cell lysates and by assessing the ability of various sigma receptor-specific ligands to compete with the radioligand for the binding (Table 1). The binding of [<sup>3</sup>H]-haloperidol (5 nM) to control cell lysates was 1.40 ± 0.07 pmol/10<sup>6</sup> cells. This value increased 3-fold to 4.33 ± 0.02 pmol/10<sup>6</sup> cells in cell lysates prepared from mSigmaR1 cDNA-transfected cells. The binding of the radioligand in cDNA-transfected cells was markedly inhibited by sigma receptor-specific ligands (clorgyline, DTC and PPP) in competition assays. These results show that the cloned mouse sigma receptor cDNA is functional.

*Isolation and restriction enzyme analysis of the murine type 1 sigma receptor genomic clone.* To isolate genomic clones containing the murine type 1 sigma receptor gene, a mouse (129Sv strain) genomic DNA library was screened using the full-length mouse SigmaR1 cDNA as the probe. Upon screening of 5 × 10<sup>5</sup> phage colonies, a single positive clone was identified and plaque-purified. The library used in this study contained the genomic DNA inserts in a phage vector with restriction sites for *SacI* and *XbaI* at both ends of the insert at the multiple cloning site. Digestion of the positive clone with *SacI* released the full-length insert of about 50 kbp in size. Digestion with *XbaI* resulted in seven fragments arising from the insert, 30.0, 6.5, 6.0, 2.7, 2.2, 0.8 and 0.5 kbp in size. Digestion with *SacI* released five fragments arising from the phage DNA (i.e., the phage arms and the genomic DNA insert) and the sizes of these fragments were 35.0, 22.0, 12.0, 7.0 and 3.5 kbp. Southern hybridization was carried out with these *SacI* and *XbaI* fragments using the full-length mSigmaR1 cDNA as the probe. This probe was found to hybridize to 2.2 and 0.8 kbp *XbaI* fragments and to the 7.0 kbp *SacI* fragment. The hybridization-positive 7.0 kbp *SacI* fragment was subcloned in pBlue-script for nucleotide sequencing.

TABLE 2  
Exon-Intron Organization of the Mouse Type 1 Sigma Receptor Gene

5' Exon			Intron			3' Exon		
Number	Size (bp)	Sequence	Donor	Number	Size (bp)	Acceptor	Sequence	Number
1	227	CAG TAT GCG C	<u>gtgagcg</u>	1	125	<u>gcaccag</u>	GC CTG GAC CAT	2
2	201	GGC CAT TCG C	<u>gtcagtg</u>	2	133	<u>caatcag</u>	CA CCA TAC TCG	3
3	93	TTC TAC CCA G	<u>gtgcgtaa</u>	3	969	<u>ctccag</u>	CA GAG ACA GTT	4
4	1072							

Lower case letters indicate intron sequences and upper case letters indicate exon sequences.



**FIG. 2.** Exon-intron organization of the murine type 1 sigma receptor gene and its organizational relationship to the mSigmaR1 cDNA. Black boxes represent the protein coding regions of the exons and the stippled boxes represent 5' untranslated region in exon 1 and 3' untranslated region in exon 4.

**Exon-intron organization of the murine type 1 sigma receptor gene.** The nucleotide sequence of the 7.0 kbp *SacI* fragment (GenBank accession no. AF 030199) was aligned with the sequence of the mSigmaR1 cDNA for exon-intron analysis and for identification of splice junctions. The gene contains four exons (227, 201, 93, and 1072 bp in size), interrupted by three introns (125, 133, and 969 bp in size). All exon-intron boundaries conform to the consensus donor/acceptor sequence (gt/ag) for RNA splicing (Table 2). The exon-intron organization of the gene and its relationship to the cDNA and the protein coding regions are depicted in Fig. 2. The locations of splice junctions are also indicated in Fig. 1. Exon 1 contains the 5'-untranslated region and part of the protein coding sequence. The splice junction between exons 1 and 2 is at the amino acid position 51/52 in the coding sequence. Exon 2 codes for the region which contains the putative transmembrane domain. Exon 3 is the shortest and is spliced to exon 2 at the amino acid position 118/119 and to exon 4 at the amino acid position 149/150. Exon 4 is the longest and contains the 3'-untranslated region and part of the protein coding sequence. The polyadenylation signal AATAAA is also present in this exon.

**Determination of the transcription start site.** The mSigmaR1 cDNA cloned from the mouse kidney cDNA library possesses a 21 bp-long 5'-untranslated region. To determine the exact transcription start site, the 5'-RACE methodology was used. The products of 5'-RACE were subcloned and several of the resultant clones were

analyzed by sequencing. Most of the clones contained a 77 bp-long 5'-untranslated region. Based on this information, the exact size of the mouse type 1 sigma receptor mRNA is 1,606 b (without the poly A tail). The clone obtained from the mouse cDNA library was short by 56 bp on the 5' end.

**Promoter analysis.** The nucleotide sequence of the 5'-flanking region of the gene is given in Fig. 3. Analysis of the sequence upstream of the ATG translation start codon using the TESS-String-Based Search of the Transcription factors Database (<http://agave.humgen.upenn.edu/utess/tess>) indicated that the classical promoter element TATA box is absent within about 100 nucleotides of the transcription start site. However, the 5'-flanking region contains a GC box (SP1 binding site) and a CCAATC box immediately upstream (nucleotide position -39 to -48) of the transcription start site. There are also consensus binding sites for AP-1, AP-2, NF-1 and GATA-1 within about 1 kbp upstream of the transcription start site. There are also several binding sites for steroid receptors (receptors for progesterone, glucocorticoids and androgens) in the 5'-flanking region. Within 1 bp consensus, a binding site for NF-GMb and a binding site for retenoic acid X receptor are also present.

In conclusion, we have cloned the murine cDNA which codes for a functional type 1 sigma receptor and used this cDNA to isolate a murine genomic clone containing the gene for this receptor. The isolated genomic clone contains the type 1 sigma receptor gene in its



-1018	TTGCTTTAGACATGGTTTCCTGAGGCTCTGCTGACTTTTGCTATGGTGA	-969
-968	CCTTGAAC TGATCTTGGTGCCTTCCCACGTTCTAGAATTACAAGCAATAC	-919
-918	ATGTCTTACATTGTCTTTTCTAACTAACATGTATAATCCTAACCATGTG	-869
-868	CCAGGCACTAATTACTTTAAAAAATTAATTCATTTAATGTTAATCATAAT	-819
-818	GAAGGTATGGGCTACGAAGCAGATACTTTTAAATTTAGTCTATGTTACAG	-769
-768	ATAAAG <u>GATA-1</u> <u>CTATCA</u> ATCCACTCTTAAATGCT <u>AP-1</u> <u>TGACACAT</u> ATGGGCTCTCTG	-719
-718	TCTTAGATCTTAGTCCAAATTTTGCT <u>SP-1</u> <u>CCCCCTCC</u> CTATTTAGCCCAGAATC	-669
-668	ATTCT <u>AP-1</u> <u>TGTGTCA</u> ACATCGAATCCCATAGCTCCCCAGTGGACAAATCTATTG	-619
-618	GTTTTCAAGGTCTTTCACACTTTTGTGCTT <u>GR/PR/AR</u> <u>AP-1</u> <u>AGAACATAGAA</u> <u>TGAATCAT</u>	-569
-568	AGGATCGGAGAAAACACTAAGCTAAACATGCATGGTGGTGC GCGCCCTG	-519
-518	ATCCCA GTATGGAGAGGCATCGATCGAGGCTATCCTAGTCTATAGGCTA	-469
-468	GCTAAAGTTGTC <u>Pit-1a</u> <u>TAAAT</u> ATTACAAATATA <u>PR/GR</u> <u>TGTACA</u> CATACATATATACA	-419
-418	TAAATGCATGTATGCTGTCTCACACTCCTCTGCTCTTT <u>AP-1</u> <u>TGACTCG</u> TGGAAT	-369
-368	AGTTC CATTTGACTCTCTCTCTCTCTCATGGACACT <u>NF-GMB</u> <u>TCAGATA</u> TCACCGC	-319
-318	TT <u>NF-1</u> <u>CTTTCC</u> TAGGTTCCCA GTGGCA <u>GATA-1</u> <u>CTATCT</u> CAACCTGCTTCTGTGCTA	-269
-268	GCAGACCTAGAAAGCTCTACTTCTGGGCTCGCTAGCTCAGGTGGGCCAGT	-219
-218	CCATTCTGGGTCTCCCCGCTT <u>AP-2</u> <u>GR</u> <u>TGGGACA</u> CCAAGATTGAACCCAACAGGGT	-169
-168	GGCAGCCAGGAGGCG <u>RXR-α</u> <u>GGCCCCGCTCT</u> CACCTCTCTTGCAGACCAAGCC	-119
-118	ACTAAACCTACGCTCTCCCTCTAGAAAATGGTTCAATCCAGAGGCGGTG	-69
-68	CTAGGTGACCCGACGTTCC <u>CCAATC Box SP1</u> <u>TGGCCCC</u> GGCAGCCGTACCACGGTGG	-19
-18	CGGGGGCGACCGTAGGCAGCGGCTTTTGAGTCGTGAACCCACAGCCTGTG	32
33	GCCCTAGTTCGGTCTTACGTTGGTGGTGCCAGGCTGCCCGCTAGAA <u>ATG</u>	80

FIG. 3. Nucleotide sequence of the 5' flanking region. The transcription start site is indicated by the nucleotide position +1, the translation start codon (ATC) for methionine is underlined and the locations of putative binding sites for several transcription factors are indicated.

entirety. We have sequenced the gene completely, deduced the exon-intron organization, and analyzed the 5'-flanking region for transcription factor binding sites. The murine type 1 sigma receptor is TATA-less and is made up of 4 exons and 3 introns. This structural information on the gene may become useful in the development of transgenic and knock-out mouse models to study the physiological significance of the receptor function and to understand the pathological consequences of the receptor dysfunction.

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#### REFERENCES

1. Itzhak, Y., and Stein, I. (1990) *Life Sci.* **47**, 1073-1081.
2. Walker, J. M., Bowen, W. D., Walker, F. O., Matsumoto, R. R., DeCosta, B., and Rice, K. C. (1990) *Pharmacol. Rev.* **42**, 355-402.
3. Ferris, C. D., Hirsch, D. J., Brooks, B. P., and Snyder, S. H. (1991) *J. Neurochem.* **57**, 729-737.
4. Su, T. P. (1991) *Eur. J. Biochem.* **200**, 633-642.
5. Quirion, R., Bowen, W. D., Itzhak, Y., Junien, J. L., Musacchio, J. M., Rothman, R. B., Su, T. P., Tam, S. W., and Taylor, D. P. (1992) *Trends Pharmacol. Sci.* **13**, 85-86.
6. Su, T. P., London, E. D., and Jaffe, J. H. (1988) *Science* **240**, 219-221.
7. McCann, D. J., and Su, T. P. (1991) *J. Pharmacol. Exp. Ther.* **257**, 547-554.
8. Ramamoorthy, J. D., Ramamoorthy, S., Mahesh, V. B., Leibach, F. H., and Ganapathy, V. (1995) *Endocrinology* **136**, 924-932.

9. Musacchio, J. M., Klein, M., and Paturzo, J. J. (1989) *Mol. Pharmacol.* **35**, 1-5.
10. DeHaven-Hudkins, D. L., Ford-Rice, F. Y., Allen, J. T., and Hudkins, R. L. (1993) *Life Sci.* **53**, 41-48.
11. Hanner, M., Moeblus, F. F., Flandorfer, A., Knaus, H. G., Striessnig, J., Kempner, E., and Glossmann, H. (1996) *Proc. Natl. Acad. Sci. U.S.A.* **93**, 8072-8077.
12. Kekuda, R., Prasad, P. D., Fei, Y. J., Leibach, F. H., and Ganapathy, V. (1996) *Biochem. Biophys. Res. Commun.* **229**, 553-558.
13. Kekuda, R., Prasad, P. D., Fei, Y. J., Torres-Zamorano, V., Sinha, S., Yang-Feng, T. L., Leibach, F. H., and Ganapathy, V. (1996) *J. Biol. Chem.* **271**, 18657-18661.
14. Kekuda, R., Torres-Zamorano, V., Fei, Y. J., Prasad, P. D., Li, H. W., Mader, L. D., Leibach, F. H., and Ganapathy, V. (1997) *Am. J. Physiol.* **272**, G1463-G1472.
15. Benson, D. A., Boguski, M., Lipman, D. J., and Ostell, J. (1996) *Nucleic Acids Res.* **24**, 1-5.
16. Eisenberg, D., Schwarz, E., Komaromy, M., and Wall, R. (1984) *J. Mol. Biol.* **179**, 125-142.
17. Schultze, M. P., Peterson, P. A., and Jackson, M. R. (1994) *EMBO J.* **13**, 1696-1705.